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Elimination and Degradation of Glucagon-like Peptide-1 and Glucose-Dependent Insulinotropic Polypeptide in Patients with End-Stage Renal Disease

Thomas Idorn, Filip K. Knop, Morten B. Jørgensen, Mikkel Christensen, Jens J. Holst, Mads Hornum, and Bo Feldt-Rasmussen

Department of Nephrology (T.I., M.B.J., M.H., B.F.-R.), Rigshospitalet, University of Copenhagen, DK-2100 Copenhagen, Denmark; Diabetes Research Division, Department of Internal Medicine (F.K.K., M.C.), Gentofte Hospital, University of Copenhagen, DK-2900 Hellerup, Denmark; and The NNF Center for Basic Metabolic Research, Department of Biomedical Sciences (F.K.K., J.J.H.), the Panum Institute, University of Copenhagen, DK-2200 Copenhagen, Denmark

Context: The affect of the kidneys in elimination and degradation of intact incretin hormones and their truncated metabolites is unclear.

Objective: To evaluate elimination and degradation of glucose-dependent insulinotropic polypeptide (GIP) and glucagon-like peptide-1 (GLP-1) in patients with dialysis-dependent kidney failure.

Setting and Design: Twelve non-diabetic patients treated with chronic hemodialysis and 12 control subjects were examined in a double-blind, randomized, matched observational study at the Department of Nephrology, Rigshospitalet, University of Copenhagen, Denmark. Over 4 separate study days, synthetic human GIP or GLP-1 was infused with or without concurrent inhibition of dipeptidyl peptidase 4 using sitagliptin or placebo. Plasma concentrations of glucose, insulin, glucagon, and intact and total forms of GLP-1 or GIP were measured repeatedly. Plasma half-life ($T_{1/2}$), metabolic clearance rate (MCR), area under curve, and volume of distribution for intact and metabolite levels of GLP-1 and GIP were calculated.

Results: Fasting concentrations of intact GLP-1 and GIP were increased in dialysis patients ($P < .001$) whereas fasting levels of GLP-1 and GIP metabolites did not differ between groups ($P > .738$). MCRs of intact GLP-1 and GIP, and the GLP-1 metabolite were reduced in dialysis patients on the placebo day ($P < .009$), and $T_{1/2}$ of intact and metabolite forms of GLP-1 and GIP were comparable between groups ($P > .121$).

Conclusions: Unexpectedly, degradation and elimination of the intact and metabolite forms of GLP-1 and GIP seemed preserved, although reduced, in patients with dialysis-dependent kidney failure. (*J Clin Endocrinol Metab* 99: 2457–2466, 2014)

The kidneys are involved in elimination and degradation of a number of hormones and drugs. The incretin hormones, glucagon-like peptide-1 (GLP-1) and glucose-

dependent insulinotropic polypeptide (GIP), are secreted from enteroendocrine L and K cells, respectively, in response to food ingestion. They stimulate insulin secretion

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Abbreviations: AUC, area under curve; BMI, body mass index; DPP-4, dipeptidyl peptidase 4; ESRD, end-stage renal disease; GIP, glucose-dependent insulinotropic polypeptide; GLP-1, glucagon-like peptide-1; MCR, metabolic clearance rate; NEP, neutral endopeptidase; $T_{1/2}$, plasma half-life.

and regulate glucagon secretion in a glucose-dependent manner (1, 2). It is widely accepted that the intact forms of the incretin hormones, GLP-1(7–36) amide and GIP(1–42), are degraded primarily by the ubiquitous enzyme dipeptidyl peptidase 4 (DPP-4) (3–5) and to a minor extent by various neutral endopeptidases (NEPs) and additional aminopeptidases (6–8). DPP-4 cleaves off the N-terminal dipeptide of both incretin hormones yielding inactive metabolites, GIP(3–42) and GLP-1(9–36) amide, respectively. These metabolites are believed primarily to be renally cleared (3–5, 9–13), although other mechanisms may contribute, including extraction in peripheral tissues (9, 14). Only a few studies have examined the effect of impaired kidney function on elimination and degradation of the incretin hormones (10–12, 15–17). Most of these studies were performed in vitro or using animal models and most were based on assays unable to distinguish between intact hormones and metabolites. Recent studies from our group have suggested a preserved ability to eliminate GLP-1 and GIP in patients with severely reduced kidney function, although only the total forms of the incretin hormones were examined (18, 19). In the present study we determined degradation and elimination of intact forms and truncated metabolites of GLP-1 and GIP in patients with chronic dialysis-dependent, end-stage renal disease (ESRD), ie patients with no or severely reduced kidney function. We hypothesized that patients with ESRD would be characterized by severe disturbances in the metabolism of both intact and, in particular, metabolite forms of the incretin hormones, caused by reduced renal function and uremia per se.

Materials and Methods

Study protocol

The study protocol was approved by the Scientific-Ethical Committee of the Capital Region of Denmark (H-2-2009-158) and by the Danish Data Protection Agency (2007-58-0015). The study was registered with clinicaltrials.gov (NCT01391884). Written informed consent was obtained and the study was conducted according to the latest revision of the Helsinki Declaration.

Subjects

We included 12 patients with ESRD (9 Caucasians, 2 Asians, and 1 African American) and 12 control subjects with normal kidney function (all Caucasians). Patients were recruited from the hemodialysis clinic at the Department of Nephrology, Rigshospitalet, Denmark. Control subjects were recruited among participants from previous projects (18, 19). Patients were matched with the control subjects according to age, sex, and body mass index (BMI). Inclusion criteria were age 18–90 y, chronic hemodialysis treatment (for ≥ 3 months), and BMI 18.5–28.0 kg/m². Patients with diabetes, pancreatitis, bowel resection, inflammatory bowel disease, malignancy (previous or present),

daily intake of medication known to influence glucose metabolism, or severe anemia were excluded. Control subjects met the same exclusion and inclusion criteria apart from having a normal kidney function (evaluated from plasma creatinine). Underlying renal diagnoses in the ESRD group included polycystic kidney disease (N = 3), interstitial nephritis (N = 2), hypertensive nephropathy (N = 2), hemolytic uremic syndrome (N = 1), amyloidosis (N = 1), congenital urinary tract malformation (N = 1), and unknown (N = 2). Concomitant pharmacological treatment in the ESRD group included weekly darbepoetin alfa and iron infusions (N = 12), daily use of vitamin substitutions and phosphorus binders (N = 12), pantoprazole (N = 7), furosemide (N = 4) and less frequently used agents (Supplemental Table 1). Glucose tolerance was assessed at an initial screening visit using a 2-h 75-g oral glucose tolerance test. One patient with ESRD had impaired glucose tolerance, 1 control subject had impaired fasting glucose, and all other participants had normal glucose tolerance (20). Nine patients with ESRD had well-treated mild-to-moderate hypertension (defined as use of antihypertensive agents and/or blood pressure $>140/90$ mm Hg at the screening visit), and one control subject had mild hypertension. Antihypertensive treatment in the ESRD group included ACE inhibitors (N = 4), angiotensin II receptor antagonists (N = 5), beta blockers (N = 3), and calcium channel blockers (N = 2). One control subject used beta blockers. Two patients with ESRD and one control subject was mildly overweight (BMI >25 kg/m²). No participants had clinically significant cardiovascular disease. Subject characteristics are presented in Table 1.

Hormone infusions, capsules, and blinding

Human GLP-1(7–36) amide and human GIP(1–42) were purchased from PolyPeptide Laboratories. The peptides were demonstrated to be greater than 97% pure and identical to the natural human peptides by HPLC, mass, and sequence analysis. The peptides were dissolved in sterilized water containing 2% human albumin (Statens Serum Institut) and subjected to duplicate sterile filtration at the Capital Region Pharmacy. Based on the molecular weights of the peptides (GLP-1(7–36) amide, 3297.67 g/mol; and GIP(1–42), 4983.68 g/mol) (21), the final GLP-1 infusion solution contained 5 μ g/mL and the GIP infusion solution contained 15 μ g/mL to be able to keep the weight-dependent infusions blinded. Infusion solutions were dispensed into glass ampoules and stored frozen (-20°C). The Capital Region Pharmacy also delivered blinded gelatin capsules containing 50 mg sitagliptin or placebo (placebo composed of lactose monohydrate, potato starch, gelatin, magnesium stearate, and talc). The products were delivered blinded with sealed randomization codes.

Experimental procedures

All participants went through 4 double-blinded examination days in randomized order: A) GLP-1 infusion + sitagliptin, B) GLP-1 infusion + placebo, C) GIP infusion + sitagliptin, and D) GIP infusion + placebo. One patient underwent kidney transplantation after 3 examination days and, consequently, the fourth examination day was not performed (GIP infusion + placebo). All other participants accomplished 4 examination days. A minimum of 72 h passed between the examination days and patients were free of dialysis treatment for greater than 36 hours before examination. Participants were examined after an overnight fast (10 h), and neither alcohol consumption nor vigorous

Table 1. Demographical and Clinical Data

	ESRD	Control	P Value ^b
Demographics			
Number, N	12	12	
Age ^a , y	44.1 ± 3.1	43.6 ± 3.9	.920
Sex, M/F	8/4	7/5	.673
BMI ^a , kg/m ²	23.7 ± 1.0	22.9 ± 0.7	.501
Caucasian, N	9	12	.217
Renal			
Arterio-venous fistula, N	12		
Dialysis duration ^a , mo	30 ± 5		
Diuresis ^a , mL/d	406 ± 213		
Dialysis adequacy ^a , Kt/V per wk	5.0 ± 0.5		
Comorbidity			
Hypertension, %	75	8	.001
Blood pressure			
Systolic blood pressure ^a , mm Hg	134 ± 5	131 ± 6	.733
Diastolic blood pressure ^a , mm Hg	86 ± 3	82 ± 3	.340
Laboratory results: screening			
Hemoglobin ^a , mmol/L	7.6 ± 0.2	8.7 ± 0.2	.001
Creatinine ^a , μmol/L	879 ± 62	66 ± 3	<.0001
Urea nitrogen ^a , mmol/L	16.3 ± 1.5	4.7 ± 0.4	<.0001
Albumin ^a , g/L	44 ± 1	45 ± 1	.756
Total carbon dioxide ^a , mmol/L	26.8 ± 1.0	27.8 ± 0.8	.489
C-reactive protein ^a , mg/L	2.8 ± 1.2	2.4 ± 1.3	.711
Hemoglobin A1c ^a , mmol/mol	33.1 ± 0.9	36.1 ± 0.9	.023
Sodium ^a , mmol/L	140 ± 1	141 ± 1	.397
Potassium ^a , mmol/L	4.6 ± 0.2	3.6 ± 0.1	.0001

Kt/V, clearance×time/volume (index).

^a Data are presented as means ± SEM.^b P values represent comparison between groups.

physical activities was permitted 24 h prior to examination. A cannula was inserted into a cubital vein for hormone infusion. In control subjects, an additional iv access (for collection of blood samples) was established from the contralateral cubital vein using an iv catheter (gauge 18), whereas blood samples were drawn from an arterio-venous fistula located at the forearm in patients with ESRD using fistula needles (gauge 17). The arm of the control subjects with the iv access for blood sampling was wrapped in a heating pad (50°C) to arterialize the blood. Each participant ingested 2 capsules containing a total of 100 mg sitagliptin or placebo 1 h prior to study start (−60 minutes). A hormone infusion, consisting of GLP-1(7–36) amide or GIP(1–42) (weight-dependent and blinded), 10 mL of 5% human albumin (CSL Behring), filled with isotonic saline (9 g/L; Amgros) to a total of 50 mL, was started at 0 minutes. The hormones were infused in doses expected to result in postprandial plasma levels (0.5 pmol GLP-1(7–36) amide/kg body weight/min and 1.0 pmol GIP(1–42)/kg body weight/min) in accordance with previous studies (10). The infusion was completed after 60 minutes and succeeded by a 2-h observational period. Venous blood samples were collected at time −15, 0, 20, 40, 60, 62, 64, 66, 70, 80, 90, 120, 150, and 180 min (frequent sampling immediately following termination of hormone infusion due to short plasma half-lives ($T_{1/2}$) of both incretin hormones). Blood was sampled into chilled tubes

containing EDTA plus a specific DPP-4 inhibitor (valine-pyrrolidide; final concentration, 0.01 mmol/L) for analyses of glucagon, GLP-1, and GIP. Tubes were immediately cooled on ice, centrifuged (1200 g for 20 min at 4°C) and stored frozen (−80°C). For analyses of insulin, blood was sampled into chilled dry tubes and left to coagulate for 30 min at room temperature before centrifugation. For bedside measurement of plasma glucose, blood was collected in tubes containing sodium fluoride and centrifuged immediately (7400 g for 2 min at room temperature).

Analyses

Following completion of all examination days, the study was unblinded in order to allocate plasma samples from days of GLP-1 infusion to GLP-1 analysis, and plasma samples from days of GIP infusion were sent for GIP analysis. Plasma samples from the days of GLP-1 infusion were assayed for total GLP-1 immunoreactivity using an antiserum that reacts equally with intact GLP-1 (GLP-1 (7–36) amide) and the N-terminally truncated metabolite (GLP-1 (9–36) amide) (22). Intact GLP-1 was measured separately using ELISA with two monoclonal antibodies; GLP-1F5 (C-terminally directed) and Mab26.1 (N-terminally directed) (23). Plasma samples from the days of GIP infusion were assayed for total GIP immunoreactivity using the C-terminally directed antiserum 867, which reacts fully with intact GIP (GIP (1–42)) and the metabolite form (GIP (3–42)) (24). Intact GIP immunoreactivity was measured separately using antiserum no. 98171, which reacts with the N-terminus of intact GIP (3). Plasma glucose concentrations were measured by the glucose oxidase method using a glucose analyzer (YSI Inc, Model 2300 STAT plus, Yellow Springs). Plasma insulin concentrations were measured using ELISA kits on a Cobas Fara robot (Roche Diagnostics GmbH). Glucagon analyses were performed as previously described (25).

Calculations and statistical analyses

Metabolite levels of the incretin hormones were calculated as the differences between total and intact levels by subtraction (3, 26). Metabolic clearance rate (MCR) was calculated using the formula, $MCR = \text{infusion rate}/([\text{hormone}]_{\text{plateau}} - [\text{hormone}]_{\text{basal}})$, where $[\text{hormone}]_{\text{plateau}}$ is the concentration at 60 min, and $[\text{hormone}]_{\text{basal}}$ is the mean concentration of baseline and 180 min. The fractional clearance rate, k , was calculated after \log_e transformation of data by linear regression analysis using the samples collected after the end of the infusion period (> 60 min). $T_{1/2}$ and the volume of distribution (V_d) were calculated using standard pharmacokinetic formulas: $T_{1/2} = \ln(2)/k$ and $V_d = MCR/k$. Area under curve (AUC) was calculated using the trapezoidal rule. Pharmacokinetic data were analyzed using GraphPad Prism software, version 6.01. A few outliers in the calculation of the fractional clearance rate, k , were detected in both groups by graphical evaluation and removed prior to further calculations. Fasting concentrations were evaluated as mean of the different examination days. Comparisons of numeric data between groups/examination days were performed using unpaired t test between groups and paired t test within groups. Fisher's Exact test was used for comparisons of categorical data between groups. Responses of plasma glucose, insulin, and glucagon were evaluated using baseline-subtracted AUCs. All tests were two-tailed and a P value <.05 was considered significant. Results are expressed as means ± SEM or means (95% confidence interval) unless otherwise stated.

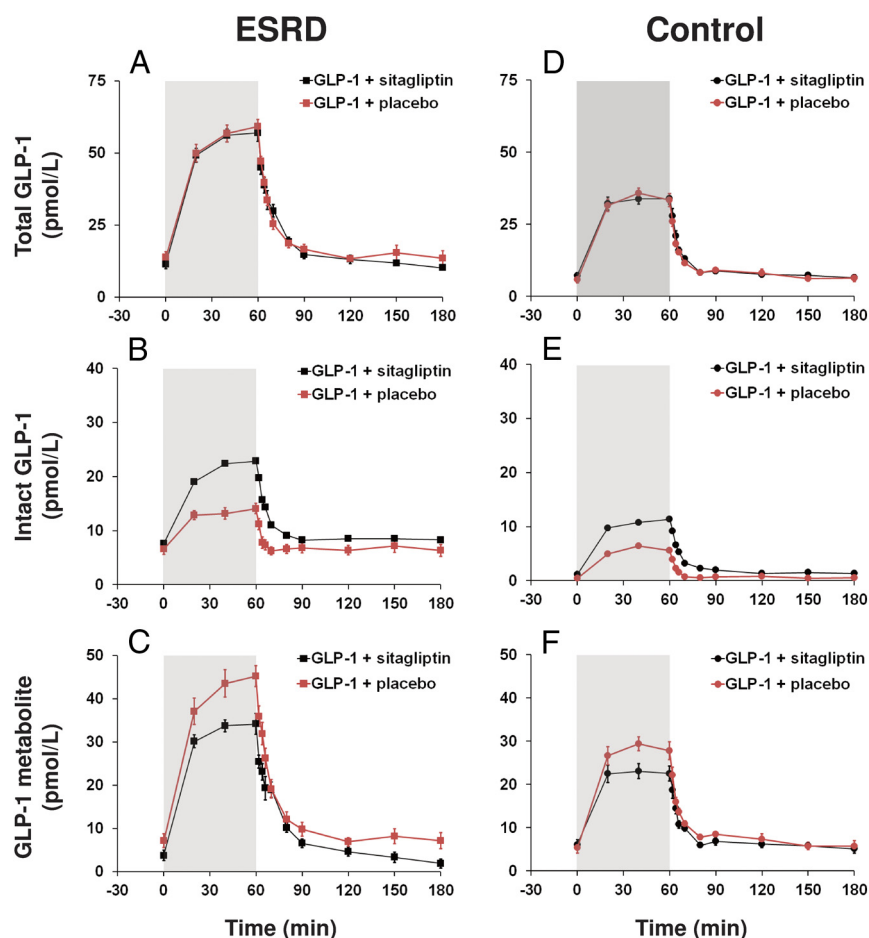


Figure 1. Plasma total glucagon-like peptide-1 (GLP-1) (A and D), intact GLP-1 (B and E), and GLP-1 metabolite (C and F) measurements during GLP-1 infusion with dipeptidyl peptidase 4 (DPP-4) inhibition (black curves) and without DPP-4 inhibition (red curves) in patients with ESRD (A–C) and control subjects (D–F). The gray bars indicate the time period for infusion (0–60 min). Data are mean \pm SEM.

Pilot study

A preceding pilot study was performed to estimate time to steady-state concentrations of intact and total forms of GLP-1 and GIP during infusion. Two patients with ESRD were examined unblinded using the same setup as described above with the exception that hormone infusions were continued until 120 min. Each patient underwent 2 examination days: GLP-1 or GIP infusion, respectively, with initial sitagliptin/placebo ingestion. Total and intact plasma concentrations of GLP-1 or GIP were measured at 17 individual time points. Using graphical evaluation, steady state was estimated to occur at 60 min for both GLP-1 and GIP. (Figures are attached as Supplemental Figure 1; only results from the main study are presented below.)

Results

GLP-1

Fasting concentrations

Fasting concentrations of intact GLP-1 (7.1 ± 0.7 pmol/L vs. 0.8 ± 0.2 pmol/L; $P < .0001$) and total GLP-1 (12.6 ± 1.2 pmol/L vs. 6.5 ± 0.8 pmol/L; $P = .001$) were

higher in patients with ESRD than in controls. Calculated fasting concentrations of the GLP-1 metabolite did not differ between groups (5.5 ± 1.0 pmol/L vs. 5.7 ± 0.8 pmol/L; $P = .811$).

Intact GLP-1 (GLP-1(7–36) amide)

MCR of intact GLP-1 was reduced in patients with ESRD compared with controls on both examination days with GLP-1 infusion ($P < .033$), while $T_{1/2}$ was similar in the two groups ($P > .383$). Baseline-corrected AUC was comparable between groups at the placebo day ($P = .536$), but significantly increased in the ESRD group following GLP-1 infusion with sitagliptin ($P = .019$).

Metabolite GLP-1 (GLP-1(9–36) amide)

MCR of the GLP-1 metabolite was significantly reduced in patients with ESRD on both examination days ($P < .007$). $T_{1/2}$ was increased in patients with ESRD compared with controls, insignificantly at the placebo day ($P = .121$) and significantly after sitagliptin ingestion ($P = .020$). AUC was significantly increased in the ESRD group at both examination days ($P < .018$) and Vd was comparable between groups ($P > .509$).

Efficacy of DPP-4 inhibition

Use of sitagliptin significantly increased $T_{1/2}$ and reduced MCR of intact GLP-1 in both groups ($P < .013$), whereas $T_{1/2}$ and MCR of the GLP-1 metabolite remained statistically unaltered by DPP-4 inhibition ($P > .255$). Curves and pharmacokinetic data are presented in Figure 1 and Table 2.

GIP

Due to previously described interference in the assay used for intact GIP analysis (3), baseline-subtracted GIP responses were used for evaluation of the pharmacokinetic parameters.

Fasting concentrations

Fasting concentrations of intact GIP (34.1 ± 2.3 pmol/L vs. 19.7 ± 1.8 pmol/L; $P < .001$) and total GIP (15.2 ± 2.4 pmol/L vs. 1.7 ± 0.4 pmol/L; $P = .001$) were

Table 2. Pharmacokinetic Parameters for GLP-1 and GIP

Parameter	GLP-1 + Sitagliptin			GLP-1 + Placebo		
	ESRD Mean (95% CI)	Control Mean (95% CI)	P Value	ESRD Mean (95% CI)	Control Mean (95% CI)	P Value
Intact GLP-1 (GLP-1(7–36 amide))						
T _{1/2} (min)	4.66 (3.84–5.92)	4.07 (3.36–5.18)	.383	2.47 (1.96–3.36) ^a	2.53 (2.03–3.36) ^c	.899
AUC (min × pmol/L)	867 (645–1089)	578 (459–697)	.019	351 (186–516) ^a	301 (241–361) ^d	.536
MCR (mL/kg/min)	36.7 (24.6–48.8)	50.5 (44.7–56.3)	.033	70.5 (60.1–80.8) ^b	105.6 (83.1–128.2) ^d	.005
Vd (L/kg)	0.25 (0.17–0.33)	0.30 (0.26–0.33)	.271	0.25 (0.21–0.29)	0.39 (0.30–0.47)	.004
Metabolite GLP-1 (GLP-1(9–36 amide))						
T _{1/2} (min)	7.83 (5.28–15.11)	3.26 (2.29–5.65)	.020	5.51 (3.99–8.91)	3.50 (2.53–5.70)	.121
AUC (min × pmol/L)	1804 (1416–2191)	926 (468–1385)	.004	2110 (1782–2439)	1430 (944–1916)	.018
MCR (mL/kg/min)	17.5 (13.7–21.3)	35.3 (23.2–47.4)	.007	13.7 (12.0–15.3)	26.7 (19.2–34.2)	.002
Vd (L/kg)	0.20 (0.16–0.24)	0.17 (0.11–0.22)	.509	0.11 (0.10–0.12)	0.13 (0.10–0.17)	.852
Intact GIP (GIP(1–42))						
T _{1/2} (min)	8.66 (5.01–31.91)	6.30 (4.08–13.80)	.477	4.47 (2.86–10.21)	3.35 (1.85–18.12)	.594
AUC (min × pmol/L)	2444 (1766–3122)	1766 (1190–2341)	.107	1523 (1097–1949) ^a	670 (369–971) ^c	.001
MCR (mL/kg/min)	31.8 (20.8–42.7)	42.6 (32.5–56.6)	.098	49.9 (34.4–67.0) ^a	99.1 (67.7–136.5) ^c	.009
Vd (L/kg)	0.40 (0.26–0.53)	0.39 (0.30–0.51)	.921	0.32 (0.22–0.43)	0.48 (0.33–0.66)	.126
Metabolite GIP (GIP(3–42))						
T _{1/2} (min)	15.34 (13.26–18.18)	10.03 (8.00–13.43)	.311	22.22 (16.18–35.46) ^a	15.77 (12.71–20.78)	.447
AUC (min × pmol/L)	1667 (860–2473)	787 (57–1518)	.089	3689 (2641–4737) ^b	2377 (1548–3206) ^d	.040
MCR (mL/kg/min)	27.6 (22.0–33.2)	36.2 (26.4–45.9)	.097	21.6 (11.5–31.7) ^b	36.7 (16.6–56.8) ^c	.163
Vd (L/kg)	0.61 (0.49–0.73)	0.52 (0.38–0.66)	.948	0.69 (0.37–1.02)	0.83 (0.38–1.29)	.346

Vd, volume of distribution.

Listed *P* values represent comparison between groups.

Footnotes represent significance levels from within-group comparisons.

^a ESRD during GLP-1/GIP infusion with sitagliptin vs. GLP-1/GIP infusion with placebo, *P* < .05.

^b ESRD during GLP-1/GIP infusion with sitagliptin vs. GLP-1/GIP infusion with placebo, *P* < .001.

^c Controls during GLP-1/GIP infusion with sitagliptin vs. GLP-1/GIP infusion with placebo, *P* < .05.

^d Controls during GLP-1/GIP infusion with sitagliptin vs. GLP-1/GIP infusion with placebo, *P* < .001.

increased in patients with ESRD compared with controls. A mean difference of 1.4 ± 4.1 pmol/L between groups of the calculated fasting concentrations of GIP metabolites did not differ significantly (*P* = .738).

Intact GIP (GIP(1–42))

MCR of intact GIP was reduced and AUC was increased in patients with ESRD compared with controls; significantly at the placebo day (*P* < .009) and insignificantly after sitagliptin ingestion (*P* > .098). T_{1/2} and Vd were similar in the two groups at both examination days (*P* > .126).

Metabolite GIP (GIP(3–42))

T_{1/2}, MCR, and Vd of the GIP metabolite were comparable between groups during GIP infusion with sitagliptin and placebo, respectively (*P* > .097), whereas AUC was increased in the ESRD group at the placebo day (*P* = .040).

Efficacy of DPP-4 inhibition

Sitagliptin administration increased T_{1/2} and reduced MCR of intact GIP in both groups, although only MCR

was changed with statistical significance (*P* < .032). Curves and pharmacokinetic data are presented in Figure 2 and Table 2.

Plasma glucose

No difference in fasting plasma glucose was observed between patients with ESRD and controls (5.1 ± 0.1 mmol/L vs. 5.2 ± 0.1 mmol/L; *P* = .476). Responses following GLP-1 and GIP infusion, respectively, with or without DPP-4 inhibition, did not differ between groups (*P* > .186). The glucose-lowering effect of GLP-1 and GIP infusion, respectively, was more pronounced during DPP-4 inhibition, although it did not reach statistical significance (*P* > .107) (Figure 3).

Insulin and glucagon

Patients with ESRD and controls exhibited comparable fasting concentrations of insulin (45.1 ± 3.8 pmol/L vs. 46.5 ± 8.6 pmol/L; *P* = .879), whereas fasting concentrations of glucagon were elevated in the ESRD group

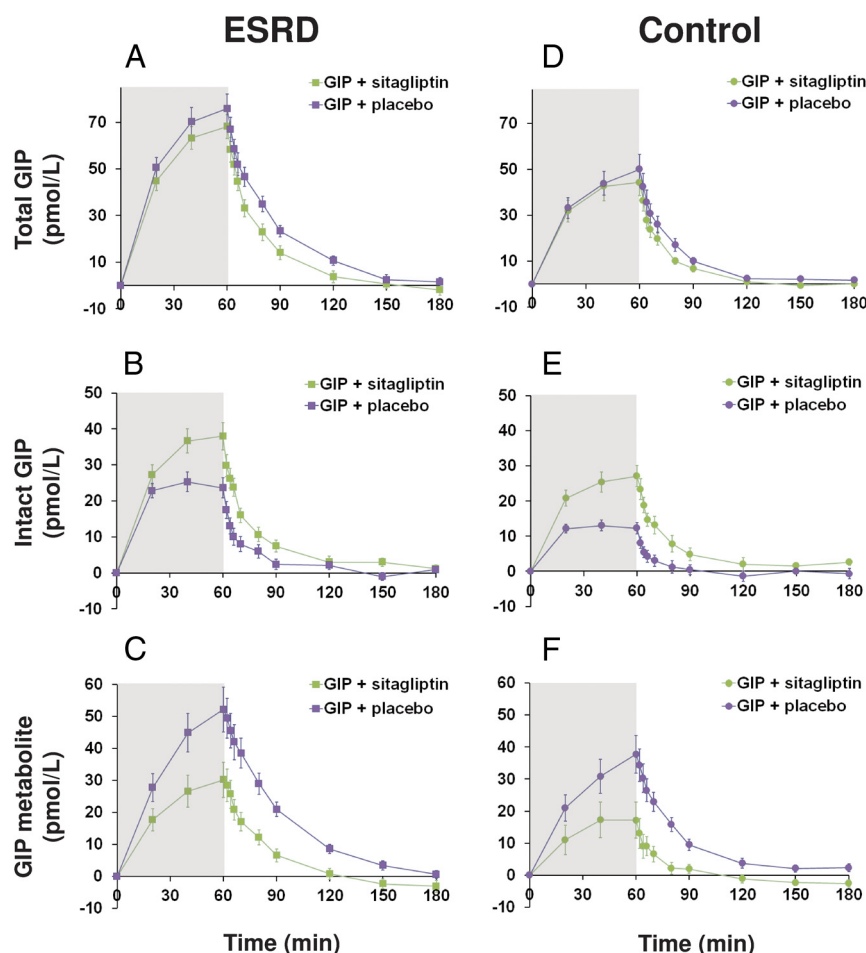


Figure 2. Plasma total GIP (A and D), intact GIP (B and E), and GIP metabolite (C and F) measurements, illustrated as baseline-subtracted values during GIP infusion with dipeptidyl peptidase 4 (DPP-4) inhibition (green curves) and without DPP-4 inhibition (purple curves) in patients with ESRD (A–C) and control subjects (D–F). The gray bars indicate the time period for infusion (0–60 min). Data are mean \pm SEM.

(25.2 ± 2.4 pmol/L vs. 6.7 ± 0.6 mmol/L; $P < .0001$). Baseline-corrected responses of insulin and glucagon were comparable between groups on all examination days ($P > .553$ and $P > .300$, respectively), with the exception that only the control group demonstrated a significant suppression of glucagon during GLP-1 infusion with sitagliptin ($P = .040$). The baseline-corrected insulin and glucagon responses were unaltered by pretreatment with sitagliptin compared with placebo in both groups ($P > .481$ and $P > .102$, respectively) (Figure 4).

Discussion

In the present study we demonstrate that non-diabetic patients with ESRD are characterized by A) preserved, although reduced degradation of GLP-1(7–36) amide and GIP(1–42), indicating a conserved function of DPP-4 and other peptidases involved in the truncation of intact incretin hormones; B) delayed, but relatively preserved

elimination of the N-terminally truncated metabolites GLP-1(9–36) amide and GIP(3–42), pointing to the importance of concurrent nonrenal elimination of these; and C) significantly increased fasting concentrations of intact GLP-1 and GIP.

The present study examined patients with chronic dialysis-dependent ESRD, ie, patients without or with only negligible renal function. The kidneys of patients with chronic ESRD consist of fibrotic tissue and very few, if any, functioning cells. Consequently, renal endocrine, exocrine, and tubular functions, clearance, and the ability to concentrate urine are abolished, and the renal blood flow is severely reduced or nonexistent (27–29). Eight of 12 patients in our study had no diuresis, indicating total cessation of any renal functions. Four patients had preserved diuresis in the range of 300–2300 mL/d. Urine from a patient with ESRD contains small amounts of waste products and electrolytes due to renal clearance close to 0 mL/min (30). Accordingly, the pharmacokinetic data did not differ between our ESRD subjects with and without diuresis ($P > .152$; MCR values for each subgroup at the placebo-days

are presented as Supplemental Table 2). Thus, the present study should enable us to elaborate on the role of the kidneys in degradation and elimination of intact and metabolite forms of GLP-1 and GIP. Sitagliptin-induced DPP-4 inhibition was included in the design in order to further visualize the effect of the kidneys on the elimination of the incretin hormones after reduction of the endogenous DPP-4-mediated degradation. The placebo day allowed us to estimate the endogenous DPP-4 activity. As expected, DPP-4 inhibition resulted in increased concentrations of intact hormone levels and, hence, reduced concentrations of the metabolites compared with placebo (Figure 1, B–F and Figure 2, B–F), resulting in a significantly reduced MCR of intact GLP-1 and GIP by approximately 50% in both groups ($P < .014$). Thus, our data suggest preserved DPP-4-mediated degradation of intact GLP-1 and GIP in patients with ESRD. To our knowledge, only one study has previously examined degradation and elimination of intact GLP-1 and GIP in patients with renal

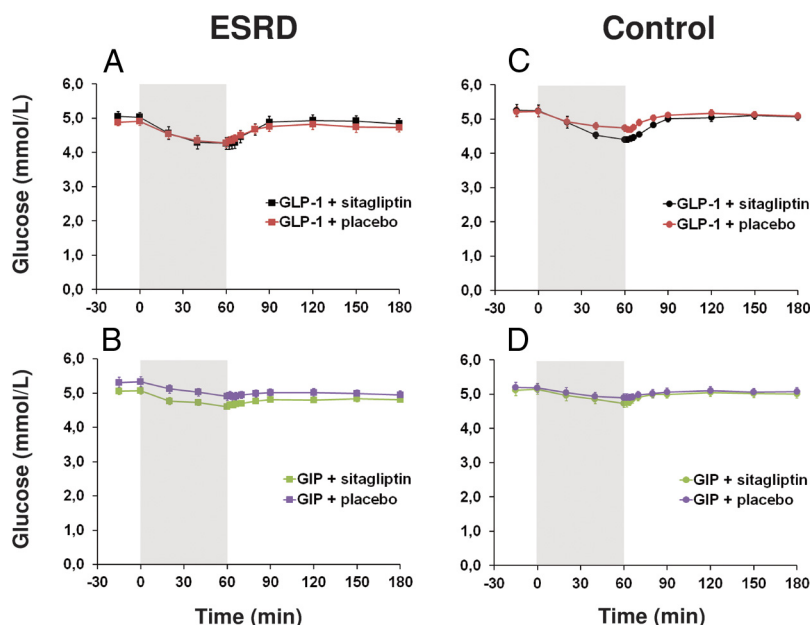


Figure 3. Plasma glucose responses during GLP-1 infusion (A and C) and GIP infusion (B and D) with dipeptidyl peptidase 4 (DPP-4) inhibition (black and green curves) and without DPP-4 inhibition (red and blue curves) in patients with ESRD (A and B) and control subjects (C and D). The gray bars indicate the time period for infusion (0–60 min). Data are mean \pm SEM.

impairment: Meier et al (10) enrolled 10 patients with moderate renal impairment (creatinine clearance 46 ± 24 mL/min) and infused GLP-1 and GIP over 30 min (ie, not sufficiently for obtaining steady state according to our pilot study (Supplemental Figure 1)). $T_{1/2}$ of intact GLP-1 has previously been reported to be approximately 2 min in healthy individuals (10, 26), and Meier et al (10) found this to be insignificantly increased in patients with moderate renal impairment (3.4 min; $P = .13$). The same pattern was observed with regard to intact GIP, and MCR of intact GLP-1 and GIP was insignificantly reduced (3, 10). These results are in accordance with our findings, although the differences were more pronounced in patients with severe renal impairment; MCR of intact GLP-1 and GIP was reduced in the ESRD group by approximately one third compared with the control group and AUCs of both intact hormones were increased. This suggests a contributory role of the kidneys in degradation of intact GLP-1 and GIP causing reduced, although partially preserved degradation in patients with ESRD. $T_{1/2}$ of intact GIP was insignificantly increased in the ESRD group, whereas $T_{1/2}$ of intact GLP-1 was almost identical in the two groups. Previous studies on pigs with normal kidney function have demonstrated a renal extraction of approximately 70% of intact GLP-1 and 25% of intact GIP with glomerular filtration, proteolysis, and perhaps peritubular reuptake being involved (9, 14). With the kidneys receiving approximately 25% of the cardiac output, approximately 10–20% of the clearance of intact incretin hormones can be explained by this mechanism. In addition to DPP-4-

mediated degradation of intact incretin hormones, extraction has been shown to occur in the liver (GLP-1 and GIP) (9, 14), extremities, and lungs (GLP-1) (14). These nonrenal degradation mechanisms probably explain the only slightly reduced ability to eliminate intact GLP-1 and GIP in the ESRD group as observed both with and without DPP-4 inhibition. In summary, our results suggest that nonrenal, non-DPP-4-mediated degradation constitutes a possible compensatory mechanism for the maintenance of approximately two thirds of normal degradation and elimination of the intact incretin hormones.

As with intact incretin hormones, few studies have examined elimination and degradation of the metabolites of GLP-1 and GIP in the setting of renal impairment. Most of these studies used assays unable to distinguish between intact hormones and N-terminally truncated metabolites (10–12, 15–17). Nevertheless, the kidneys have been suggested to be crucial for the elimination of “total GLP-1” (10–12) and “total GIP” (10, 15–17). Meier et al (10) measured intact and metabolite levels separately and concluded, in accordance with previous studies, that the kidneys are essential for elimination of the metabolites of GLP-1 and GIP. Nonetheless, elimination and degradation of the N-terminally truncated metabolites have never been examined in patients with ESRD. The present study clearly reveals involvement of the kidneys in elimination and degradation of the metabolites of GLP-1 and GIP; MCR values were reduced by 40–50% in the ESRD group compared with the control group during the placebo days and AUCs were increased. It is, however, surprising that nonrenal degradation and elimination can partly compensate for the extinct or severely reduced kidney function, which is in contrast to previous studies (10–12, 15–17). The insignificantly increased $T_{1/2}$ of both metabolites in patients with ESRD and the comparable fasting concentrations between groups strengthen this conclusion. Increased activity of NEPs may constitute a possible explanation. NEP 24.11 (nepriylsin, CD10) is the most commonly occurring and well studied of these enzymes and has been detected in several tissues and organs. It cleaves GLP-1 and GIP at various sites, resulting in metabolites different from the DPP-4-induced cleavage products (i.e. metabolites that are not caught in the assay measuring total GLP-1 and GIP

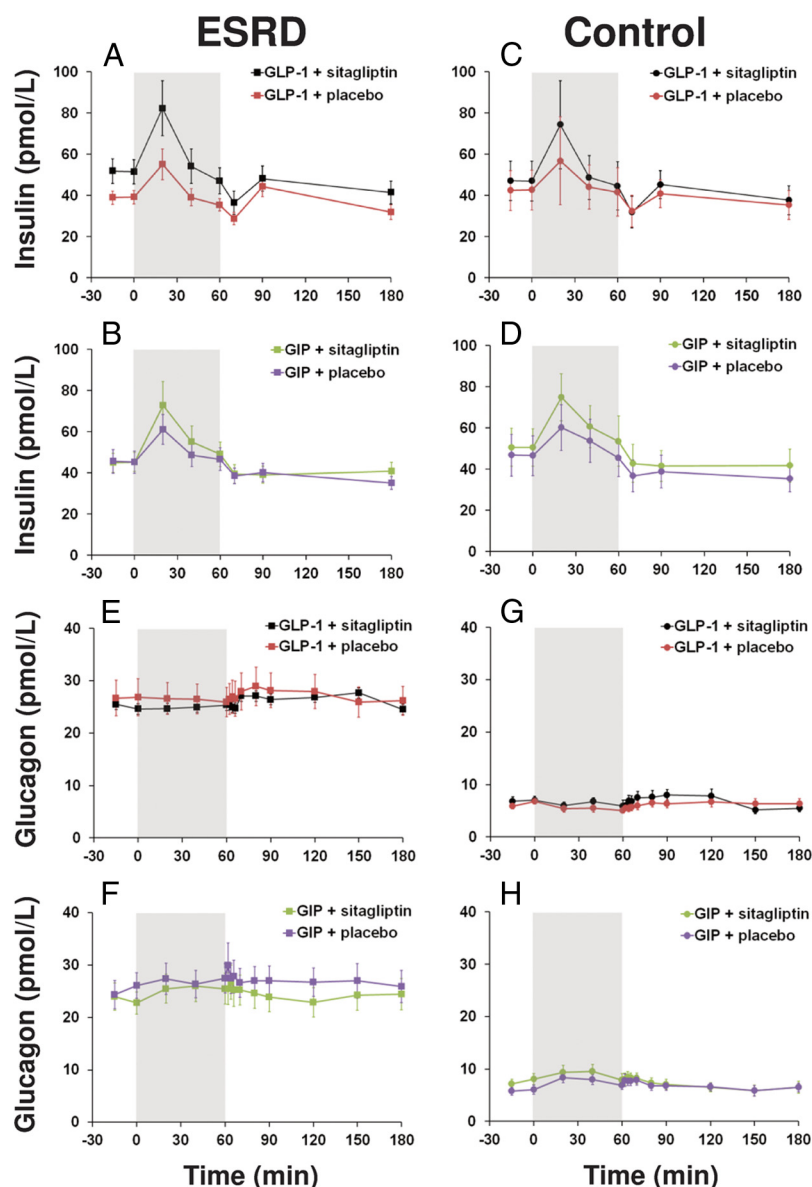


Figure 4. Plasma insulin (A–D) and glucagon (E–H) responses during GLP-1 infusion (A, C, E, and G) and GIP infusion (B, D, F, and H) with dipeptidyl peptidase 4 (DPP-4) inhibition (black and green curves) and without DPP-4 inhibition (red and blue curves) in patients with ESRD (A, B, E, and F) and control subjects (C, D, G, and H). The gray bars indicate the time period for infusion (0–60 min). Data are mean \pm SEM.

in our study) (6, 7, 13). NEP 24.11 degrades GLP-1 both in vivo and in vitro (6, 7, 31). GIP is degraded more slowly by NEP 24.11 in vitro and its relevance in GIP metabolism in vivo has not yet been established (6, 7, 32). Organ extraction constitutes another possible compensatory mechanism for degradation and elimination of the truncated metabolites. A significant extraction from the fibrotic kidneys in patients with ESRD is unlikely (27–30) and previous studies measuring total levels of GLP-1 (14) and GIP (33) have suggested that the liver does not play a major role in the elimination and degradation of the metabolites of the incretin hormones. Both N- and C-terminal degradation of the incretin hormones occurs in peripheral tissues

(9, 14), thus constituting a possible nonrenal compensatory elimination site. Extraction from other organs cannot be excluded and additional studies examining in vivo and in vitro organ-specific extraction as well as NEP activity in patients with varying degrees of renal impairment are warranted.

The presence of significantly increased fasting concentrations of intact GLP-1 and GIP in patients with ESRD represents novel findings. Causes and/or consequences cannot be extracted from the present study, but it correlates well with the results of a previous study in which patients with ESRD exhibited exaggerated postprandial responses of total GLP-1 compared with healthy control subjects (19). Furthermore, our data confirm the presence of severely increased fasting concentrations of glucagon in nondiabetic patients with ESRD (18, 19), the underlying pathophysiology of which is yet uncharted. Fasting glucose and insulin concentrations were comparable and within normal levels in both groups, suggesting preserved insulin sensitivity. Even though insulin resistance is common in patients with ESRD (34), this finding is in line with previous studies by our group (18, 19). The negligible responses of insulin and glucagon and the corresponding, limited effects on plasma glucose in the present study are probably due to the normoglycemic experimental conditions, given that

neither GLP-1 nor GIP induce major changes in islet secretion at normoglycemia. Future studies using clamp techniques during GLP-1 and GIP infusion, respectively are warranted for a thorough characterization of the pancreatic effects of incretin hormones in patients with ESRD. Safety and efficacy of incretin-based therapies in patients with impaired kidney function, including patients with ESRD (35), has been investigated in randomized controlled trials. Although our study was designed solely for pharmacokinetic analyses, our results suggest a maintained efficacy of DPP-4 in a severe uremic environment, which would be a prerequisite for use of DPP-4 inhibitors.

Furthermore, our data may suggest the need for dose reduction of GLP-1 receptor analogs (in case these are degraded and eliminated with similar kinetics as human GLP-1) in diabetic patients with ESRD due to reduced MCR of intact and metabolite GLP-1. The delayed metabolism of the GLP-1 metabolite in patients with ESRD may have beneficial cardiovascular effects (36), although additional studies are needed to elucidate this.

Our study has limitations. We were unable to perform a regular prestudy power analysis because no historical data on the pharmacokinetics of GLP-1 and GIP in patients with severely reduced kidney function exists. Thus, type 2 errors cannot be precluded, although the number of examined patients is above that in similar pharmacokinetic human studies. Also, the patients with ESRD comprised a heterogeneous group with different underlying renal diseases and ethnicity. To minimize confounding effects of this we matched patients and controls on age, sex, and BMI and gave preference to nondiabetic patients with ESRD with little comorbidity. We assess these minor differences to be of little significance for the pharmacokinetic evaluation of incretin hormones in patients with ESRD. The study design represents a nonphysiological setting and first-pass metabolism from the portal system is not achieved following iv infusion. This may affect the pharmacokinetic results; however, previous animal studies have not identified the liver as a major site of degradation and elimination of GLP-1 and GIP (except for hepatic DPP-4 activity). Furthermore, study conditions were identical in all participants, thus allowing comparison between groups. Blood samples were drawn from a peripheral vein in control subjects whereas an arterio-venous fistula was used in the patients with ESRD. To minimize possible differences, a heating pad was used to “arterialize” the blood in the control group. Finally, we did not measure DPP-4 activity or plasma concentrations of sitagliptin. Nevertheless, the intention of the design was, qualitatively, to examine the efficacy of DPP-4 in a uremic environment and to elucidate DPP-4-independent elimination and degradation mechanisms further. These aims were achieved.

In conclusion, our results suggest that degradation and elimination of intact and N-terminally truncated metabolites of GLP-1 and GIP, respectively, are delayed, but relatively preserved, despite an extinct or severely reduced kidney function. DPP-4-mediated degradation of the intact hormones seems unaffected by severe uremia whereas nonrenal degradation and elimination of the incretin metabolites—perhaps constituted by NEPs or yet unknown factors—seem to exist.

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Address all correspondence and requests for reprints to: Thomas Idorn, Department of Nephrology, Rigshospitalet, Blegdamsvej 9, DK-2100 Copenhagen, Denmark. E-mail: thomas.idorn@regionh.dk.

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